Global Advanced Research Journal of Biochemistry and Bioinformatics Vol. 2(x) pp. xxx-xxx, January, 2013
Available online http://garj.org/garjbb/index.htm

Original Research Paper

IL-4 Variable number of tendon repeats (P2/P2 genotype) is associated with increased risk of prostate cancer

Nega Berhane[1]*, Shiferaw Melesse[2]

[1]Department of Biotechnology, University of Gondar, Gondar, Ethiopia
[2]Department of Mathematics, University of Gondar, Gondar, Ethiopia

Accepted 15th January 2013

The etiology of Prostate Cancer (PCa) is largely unknown. Several factors such as ethnicity, family history, and age have been shown to be associated with increased risk of Prostate Cancer. There is emerging evidence that polymorphic genes may modulate effects of endogenous androgens or environmental toxicants on Prostate Cancer risk. There have been reports that polymorphisms in the promoter regions of cytokine genes may influence prostate cancer development via regulation of the antitumor immune response and/or pathways of tumor angiogenesis. *IL-4* an anti-inflammatory cytokine plays a key role in activation and differentiation of B-cells, mast cells, erythroid progenitors and the development of the Th-2 subset of lymphocytes. Th2 cytokines such as *IL-4*, *IL-2* and *IL-10* primarily supports antibody production. IL-4 is also known to inhibit macrophage activation and therefore may be involve in cancer. In this case control study 150 prostate cancer patients and equal number of age matched two control groups that includes, one hundred fifty individuals diagnosed with Benign prostate hyperplasia (BPH) and 150 health individuals were recruited. The objective of this study was to evaluate the effect of *IL-4* VNTR on risk of prostate cancer among north Indian population. The PCR (polymerase chain reaction) were utilized to identify the three genotypes of *IL-4* VNTR gene. Statistically significant increased risk of prostate cancer was associated with individuals that carry the *p2/P2* genotypes (OR=2.45 95%CI=0.98-6.23, *p*< 0.05).

Keywords: Cytokine; polymorphisms; *IL-4* VNTR; Prostate cancer; Benign hyperplasia

INTRODUCTION

Prostate cancer, unlike cancers of other sites, often has a very indolent natural history. Whereas patients with any other curable cancer would automatically be offered radical treatment, “watchful waiting” has been a recognised approach to managing prostate cancer, with acceptable results in selected patients (Albertsen *et al.,*1998). Prostate cancer (PCa) is the most common noncutaneous cancer among men, accounting for 10% of male cancer-related death (Jemal *et al.,* 2009). The etiology of PCa is largely unknown, although multiple environmental and lifestyle factors such as ultraviolet irradiation, smoking, and diet might increase the risk of the disease (Grant, 2004). However, not all of those who have been exposed to these risk factors will develop PCa, suggesting interindividual differences in susceptibility. These differences could, in part, be

*Corresponding Author’s E-mail: tesnega@yahoo.com

caused by genetic variations, such as single nucleotide polymorphisms in the DNA repair gene that increase susceptibility to the DNA damage resulting from carcinogens (Goode *et al.,* 2002). Prostate cancer is a common condition worldwide. Different geographical regions have varying incidence and mortality. Globally, prostate cancer is the sixth most common cancer, and the third most common cancer in men in developed countries. The risk of prostate cancer is increased by African-American ethnicity, increasing age, positive family history, and other factors such as diet. Nonetheless, the causes of prostate cancer are not well understood compared with other common cancers like lung and breast cancer (Kumar *et al*., 2004). There have been reports that polymorphisms in the promoter regions of cytokine genes may influence prostate cancer development via regulation of the antitumor immune response and/or pathways of tumor angiogenesis (McCarron *et al.,*2002). Single nucleotide polymorphisms or SNPs and variable number of tandem repeats (VNTR) are common type of polymorphisms. The variants of DNA sequence can have a major impact on how humans respond to disease. Moreover environmental insults such as bacteria, viruses, toxins, and chemicals; may increase the risk of PCa. SNPs do not cause disease, but they can help determine the likelihood that a particular individual may develop a particular disease (Kesarwani *et al.,* 2008). *IL-4* an anti-inflammatory cytokine plays a key role in activation and differentiation of B-cells, mast cells, erythroid progenitors and the development of the Th-2 subset of lymphocytes. Th2 cytokines such as *IL-4*, *IL-2* and *IL-10* primarily supports antibody production. *IL-4* is also known to inhibit macrophage activation and therefore, may be involve in cancer. A variable number of tandem repeat (VNTR) of 70 base pair repeat is situated in third intronic region of the *IL-4* gene. Three repeat allele is most common and two repeat allele is rare. There is another much rare allele of four repeat, which is reported only in few populations (Kesarwani *et al.,* 2008). Three repeat allele is known to be high producer of IL-4 (Nakashima *et al*., 1999). The *IL-4* gene is located on the long arm of chromosome 5 (q23–31) in a cluster of other cytokine genes (*IL-3*, -5, -9, -13, and -15, granulocyte colony-stimulating factor, and interferon regulatory factor) (Le Beau *et al*., 1998). It is also a key T helper-2 cytokine that downregulates and upregulates CCR5 and CXCR4 (Chemochine receptors), respectively, the main coreceptors for HIV (Soruano *et al*., 2005). Earlier studies have reported that *IL-4* inhibits the release of inflammatory mediators, such as *TNF-α*, *IL-6*, and *IL-1α* from activated monocytes **(**Weiss *et al*., 2003; Mittal & Manchanda, 2007). Interleukin-4 is a key cytokine that induces the activation and differentiation of B cells, and the development of the Th2 subset of lymphocytes. Th2 cytokines such as interleukin-4, 6 and 10 primarily support antibody production, and many studies have confirmed that patients with cancer have high levels of such cytokines in their serum. Interleukin-4 also inhibits macrophage activation and might be involved in cancer causations (Sosroseno *et al*., 1994; Wang *et al*., 2002). Thus, the aim of the present study was to assess the effect of this VNTR on the risk of PCa among north Indian population.

MATERIALS AND METHODS

Characteristics of study subjects

Blood samples from 150 north Indian males with prostate cancer were collected in sterile EDTA-K2 coated non-vacationous tubes (Becton–Dickinson, San Jose, CA, USA). The samples were collected at the Departments of Urology of the Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarh, India, the All India Institute of Medical Sciences (AIIMS), New Delhi and Government Medical College and Hospital, Patiala, Punjab in north India. All patients were histological diagnosed carcinoma of the prostate confirmed with biopsy studies as well as prostate specific antigens (PSA) test. There were 300 age matched control samples, of which 150 were free from any symptom of disease, whereas the rest 150 were diagnosed with benign prostate hyperplasia (BPH). All the samples were not originated from the same hospital; however, all of the individuals recruited for this study were in the same geographical area. None of the patients were given either chemo or radiotherapy before providing the sample. The samples were collected by the clinical staff of the hospitals concerned. A detailed questionnaire designed by the Indian Council of Medical Research (ICMR) encompassing details of the disease diagnosis, family history, age, smoking and drinking status was completed at the time of collection of the samples. Besides, the pathological grading and staging of the cancer was confirmed from the hospital record. Blood samples were stored at -80 ^{0}C till extraction of DNA. Written informed consent was obtained from all cases and controls. The study was carried out after obtaining approval from the Ethics Committee of the PGIMER, Chandigarh, India.

Genotyping of IL-4 VNTR

Genomic DNA was extracted from peripheral blood lymphocytes by standardized protienase K digestion and phenol– chloroform extraction (Sa´enz-Lo´pez *et al.,* 2008). The *IL-4* VNTR was studied by using the polymerase chain reaction. A 70 bp variable number tandem repeat (VNTR) of *IL- 4* gene was amplified by using primers; forward 5'-AGGCTGAAAGGGGGAAAGC-3' and reverse 5'-5'-CTGTTCACCTCAACTGCTCC-3' as described by Kesarwani *et al.* (2008). The PCR amplification was

performed in 25µl volume containing 100ng of genomic DNA, 0.2mM of mixed dNTP, 20p mol of each primer, 1.5 U Taq polymerase , 10X KCl, 1.5mM $MgCl_2$ and 19µl of distilled water. The PCR conditions were 1 cycle of dnaturation 1 at 95^0C for 5 minutes, 30 cycles of denaturation 2 at 95 ^{0}C for 45 seconds followed by 1 cycle of annealing at 58^0C for 45 seconds, 72^0C for 45 seconds, 72^0C for 2 minutes, 1 cycle each for extension 1 and 2, respectively, that generated 184 bp designated as *P1/P1* and 254 bp for *P2/P2* genotypes (Fig 1).

Statistical analysis

Relevant data of cases, BPH and health controls such as age, inhabitancy, occupation, smoking and drinking habits were tabulated. The effect and association of the *IL-4* VNTR polymorphism on risk of PCa was analyzed by computing odds ratio (OR) and 95 % confidence interval (CI). The statistical analysis was performed using Epi-Info software (Epi-Info, version 3.5.1. Center for Disease Control and Prevention, Atlanta, GA, USA, August 13, 2008) and software SPSS version 11.5 (SPSS, Chicago, IL). Chi-square (χ^2) test was utilized to check for the Hardy–Weinberg's equilibrium. Significance was set at $p<0.05$.

RESULTS

The demographic characteristics of study subjects were described elsewhere (Berhane, *et al.,*2010). The genotype frequency of *IL-4* VNTR in cases, controls and BPH study subjects is given in Figure 2. The frequency of the *P1/P1* repeat in cases was 54.7% against 62.7% in controls. On the other hand, the frequency of the heterozygous *P1/P2* genotype in cases was 33.3 % unlike 31.3 % in controls. The percentage of the rare 2 repeat homozygous *P2/P2* genotype in cases was 12.7 against 6 in controls. The *P1* allelic frequency for cases and controls was 0.70 and 0.78, respectively. The *P2* allelic frequency was 0.3 in cases and it was 0.22 in controls. *P2* allele was associated with statistically increased risk for prostate cancer (OR= 1.50, 95 %CI= 1.02-2.21, p=0.039).

The number of smoker and non smoker cases, controls and BPH study subjects is given in Figure 3. The OR and 95% CI of smoking cases were computed with non smoking healthy controls. There was statically no significant association between genetic polymorphism of *IL-4* VNTR smoking and risk of prostate cancer. However, statistically non significant risk of prostate cancer on smoker cases was associated on *P2/P2* genotype carriers (OR=1.88, 95%CI=1.15-3.06)

The effect of alcohol in relation to *IL-4*VNTR polymorphism in cases, controls and BPH study subjects is given in Fig. 4. Statistically nonsignificant 1.65 folds of increased risk for prostate cancer was associated due to *P2/P2* genotype and alcoholism.

DISCUSSION

IL-4 is a prototypic member of Th2 cytokines and is a potent anti-inflammatory. It reduces the production of proinflammatory cytokines and destructive enzymes by monocytes (Chomarat *et al.,* 1995). The *IL-4* gene is located on the long arm of chromosome 5 (q23–31) together with other Th2 cytokine genes and is present in a cluster of cytokine genes (IL-3, -5, -9, -13, and -15, granulocyte colony-stimulating factor, and interferon regulatory factor; (Le Beau *et al.,* 1989). This gene contains a variable number of tandem repeat polymorphism located in third intron VNTR; (Weiss *et al.,* 2004). It consists of three 70-bp repeats in intron-3, a rare allele with two repeats and much rarer with four repeats. It is a growth costimulator for B and T cells, mast cells, erythroid progenitors, and myeloid progenitors. Previous studies reported that IL-4 inhibits the release of inflammatory mediators, such as TNF-α, IL-6, and IL-1α from activated monocytes. *IL-4* VNTR gene polymorphism is reported to be associated with rheumatoid arthritis and *IL-4* C-589T with asthma (Buchs *et al.,* 2000; Walley and Cookson, 1996). In the present study the P2 allele of IL-4 VNTR was associated with increased risk of prostate cancer risk (OR= 1.50 95% CI=1.02-2.21, p <0.05).

IL-4 serves as growth co-stimulator for a variety of cells, at the same time inhibiting the release of inflammatory mediators such as TNF-α, IL-6 and IL-1α from activated monocytes (Chomarat *et al.,* 1995). IL-4 has been reported to regulate growth and proliferation of different prostatic cells.

Associations between the 70 bp VNTR allele of IL-4 and severity of several diseases have been reported (Nakashamya *et al.,* 2002). As far as our knowledge goes there is no report on *IL-4* VNTR and risk of prostate cancer, however, Konwar *et al.* (2008) reported the *IL-4* polymorphism showed significant association with BPH, indicating that this gene is likely to be associated with this disease. In addition, they found a significant association of *IL-4* variants with patient responsiveness to combined therapy of α-adrenergic blocker 5-α reductase inhibitors. This indicated that *IL-4* polymorphism is not only associated in the susceptibility of BPH, but also influences drug responsiveness of BPH patients, with some yet unknown mechanism.

It has been previously shown that *IL-4* promotes the Th2 response and is directly involved in the pathogenesis of GN (Furusu *et al.,* 1997; Nakajima *et al*., 1997). Further, Singh *et al*. (2003), in their study, suggested that IL-4 might be directly involved in the development of lupus nephritis, particularly glomerulosclerosis and chronic renal fibrosis. Moreover, Mittal *et al.* (2004) reported that *IL-4* VNTR was

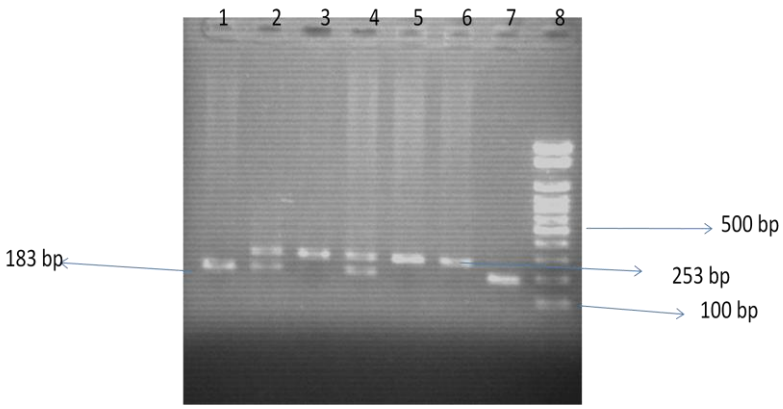

Fig.1: PCR- VNTR representative agarose gel picture of IL-4 gene of HIV seropositive subjects
Lane 8 100 bp DNA Marker
Lanes 1 and 7 P1/P1 183 bp
Lanes 2 nad 4 P1/P2 183 and 253 bp
Lanes 3,5, and 6 P2/P2 253 bp

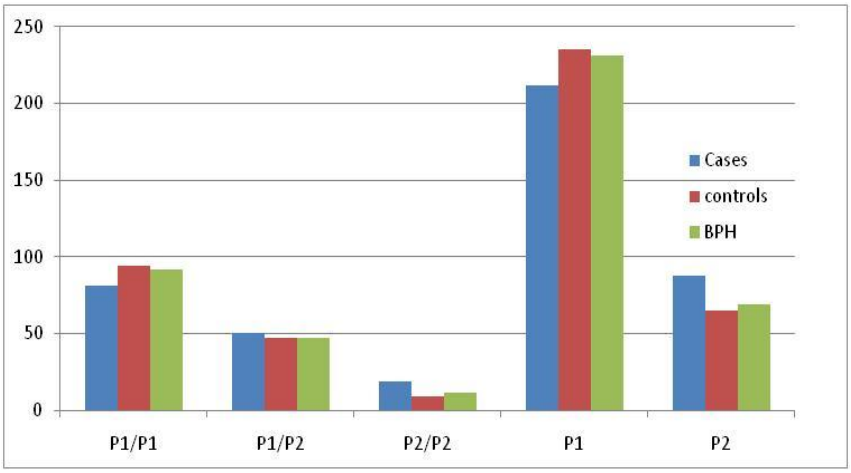

Figure 2. Frequency of the P1/P1, P1/P2/, P2/P2 genotypes and P1 and P2 alleles of IL-4 gene among cases, controls and BPH study subjects.

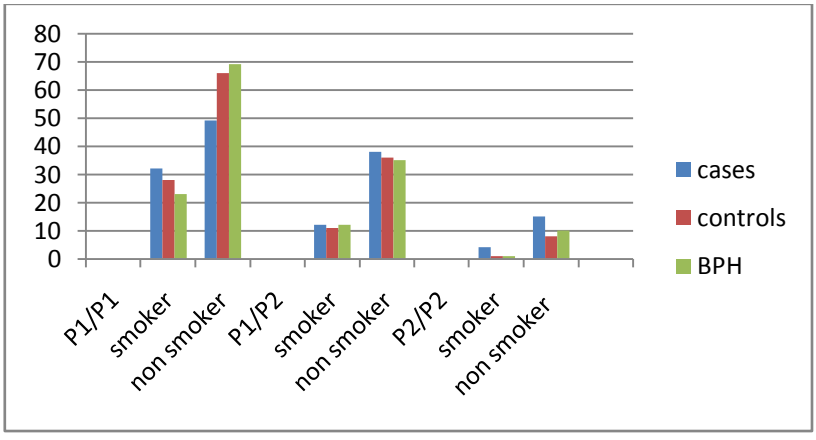

Figure 3. The genotype frequency of IL-4 VNTR and smoking status among cases, controls and BPH study subjects

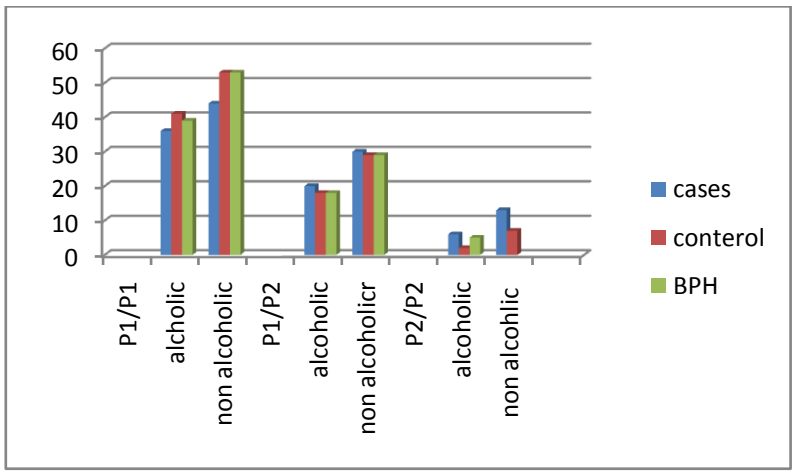

Figure 4. The genotype frequency of IL-4 VNTR and alcoholic status among cases, controls and BPH study subjects.

associated with increased risk of end stage renal disease. Regarding smoking and alcoholism no additional statistically significant association was observed in cases. To exactly indicate the *P2/P2* genotype of *IL-4* gene as a risk factor for prostate cancer additional study with large sample size in the same population and with different genetic background is warranted. Moreover, the role of IL-4 anti inflammatory cytokine in PCa patients needs to be explained by measuring IL-4 level in the serum of patients.

ACKNOWLEDGEMENT

The authors of this article are very much thankful to clinicians of the three respective hospitals at the department of Urology for providing clinical samples and valuable data about patients and controls. Moreover, the authors would like to extend their kind appreciation for Professor R.C. Sobti at the department of Biotechnology, Panjab university, Chandigarh for supporting this work with all resources required and allowing his laboratory for the practical activity.

REFERENCES

Albertsen K, Gronbaek M (2002). Does amount or type of alcohol influence the risk of prostate cancer? *Prostate* 52:297–304

Albertsen PC, Hanley JA, Gleason DF (1998). Competing risk analysis of men aged 55 to 74 years at diagnosis managed conservatively for clinically localized prostate cancer. JAMA 280: 975–80.

Berhane N, Sobti RC, Mahdi SA (2012). DNA repair genes polymorphism (XPG and XRCC1) and association of prostate cancer in a north Indian population. *Mol Biol Rep.* 39(3):2471-2479.

Buchs N, Silvestri T, di Gioveine FS (2000). IL-4 VNTR gene polymorphism in chronic polyarthritis. The rare allele is associated with protection against destruction. *Rheumatology* 39:1126–31.

Chomarat P, Vannier E, Dechanet J, Rissoan MC, Banchereau J, Dinarello CA, Miossec P (1995). Balance of IL-1 receptor antagonist/IL-1 beta in rheumatoid synovium and its regulation by IL-4 and IL-10. *J Immunol* 154**:**1432–1439

Furusu A, Miyazaki M, Koji T, Abe K, Ozono Y, Harada T, Nakane PK, Hara K, Kohno S (1997). Involvement of IL-4 in human glomerulonephritis: an in situ hybridization study of IL-4 mRNA and IL-4 receptor mRNA. *J Am Soc Nephrol* 8:730–741.

Goode EL, Ulrich CM, Potter JD (2002). Polymorphisms in DNA repair genes and associations with cancer risk. *CancerEpidemiol Biomarkers Prev* 11: 1513-1530.

Goode EL, Ulrich CM, Potter JD (2002). Polymorphisms in DNA repair genes and associations with cancer risk *CancerEpidemiol Biomarkers Prev* 11: 1513-1530

Grant WB (2004). A multicountry ecologic study of risk and risk reduction factors for prostate cancer mortality. *Eur Urol*. 45:271-279

Jemal A, Siegel R, Ward E, Murray T, Xu J, Smigal C, Thun MJ (2008). Cancer statistics, *CA Cancer J Clin*. 58:71-96.

Kesarwani P, Ahirwar D, Singh R, Manchanda PK, D Mittal RD (2008). Do IL-4 Intron 3 VNTR and IL-6 (-174) G/C Variants Reflect Ethnic Variation? A Comparative Study between the Global and North Indian Populations. *Asian Pacific Journal of Cancer Prevention* 9:76-80.

Kumar R, Barqawi A, Crawford ED (2004) Epidemiology of prostate cancer. US *oncology review.* 1: 1-6.

Le Beau MM, Lemons RS, Espinosa R, Larson RA, Arai N, Rowley JD (1998). Interleukin-4 and interleukin-5 map to human chromosome 5 in a region encoding growth factors and receptors and are deleted in myeloid leukemias with a del (5q). *Blood* 73:647–650.

McCarron SL**,** Edwards S, Evans PR, Gibbs R, Dearnaley DP, Dowe A, Southgate C, Easton DF, Eeles RA, Howell WM (2002). Influence of Cytokine gene polymorphisms on the development of prostate cancer. *Cancer Res* 62: 3369–3372.

Mittal RM, Manchanda PK (2007). Association of interleukin (IL)-4 intron-3 and IL-6 −174 G/C gene polymorphism with susceptibility to end-stage renal disease. *Immunogenetics* 59:159–165

Mout R, Willemze R, Landegent JE (1991). Repeat polymorphisms in the interleukin-4 gene (IL4). *Nucleic Acids Res* 19:3763

Nakajima A, Hirose S, Yagita H, Okumura K (1999). Roles of IL-4 and IL-12 in the development of lupus in NZB/W F1 mice. *J Immunol* 158:1466–1472.

Nakashima H, Miyake K, Inoue Y, Shimizu S, Akahoshi M, Tanaka Y, Otsuka T, Harada M (2002). Association between IL-4 genotype and IL-4 production in the Japanese population. *Genes Immun*. 3(2): 107-109.

Sa´enz-Lo´pez P, Carretero R, Co´zar JM, Romero JM, Canton J, Vilchez JR, Tallada M, Garrido F, Ruiz-Cabello F (2008). Genetic polymorphisms of RANTES, IL1-A, MCP-1 and TNF-A genes in patients with prostate cancer. *BMC Cancer* 8:382

Singh R, Eeles, R A, Durocher F, Simard J, Edwards S, Badzioch M, Kote-Jarai Z, Teare D, Ford D, Dearnaley D, Arden-Jones A, Murkin

A, Dowe A, Shearer R, Kelly J (2003). The CRC/BPG UK Familial
Prostate Cancer Study Collaborators, High risk genes predisposing to prostate cancer development–do they exist? *Prostate Cancer and Prostatic Diseases* 3:241–247.

Soriano A, Lozano F, Oliva H, García F, Nomdedéu M, De Lazzari E, Rodríguez C, Barrasa A, Lorenzo JI, Del Romero J, Plana M, Miró JM, Gatell JM, Vives J, Gallart T (2005). Polymorphisms in the interleukin-4 receptor *α* chain gene influence susceptibility to HIV-1 infection and its progression to AIDS. *Immunogenetic* 57:644–654.

Sosroseno W, Herminajeng E, Goeno S (1994). The interleukin net work in the immunopathegenesis of oral disease. *Asian Pac J Allergy Immunol*. 12**:** 161-168

Sosroseno W, Herminajeng E, Goeno S (1994). The interleukin net work in the immunopathegenesis of oral disease. *Asian Pac J Allergy Immunol*. 12**:** 161-168.

Walley AJ, Cookson WO (1996). Investigation of an interleukin-4 promoter polymorphism for associations with asthma and atopy. *J Med Genet.* 33:689–692

Wang CY, Debiec-Rychter M, Schut HA, Morse P, Jones RF, Archer C, King CM, and Haas GP (1999). N-Acetyltransferase expression and DNA binding of N-hydroxyheterocyclic amines in human prostate epithelium. *Carcinogenesis.* 20*:* 1591–1595.

Wang Y, Spitz MR, Zhu Y, Dong Q, Shete S, Wu X (2003). From gene type to phenotype: correlating XRCC1 polymorphisms with mutagen sensitivity. *DNA Repair Amst*. 2:901-908.

Wang YF, Chang SY, Tai SK, Li WY, Wang LS (2002). Clinical significance of interleukin-6 and interleukin-6 receptor expressions in oral squamous cell carcinoma. *Head Neck*. 24:850–858

Weiss T, Shalit I, Blau H, Werber S, Halperin D, Levitov A, Fabian (2004). Anti-Inflammatory Effects of Moxifloxacin on Activated Human Monocytic Cells: Inhibition of NF-κB and Mitogen-Activated Protein Kinase Activation and of Synthesis of Proinflammatory Cytokines. *Antimicrob Agents Chemother* 48(6): 1974–1982.